

Studies of the Genus *Bupleurum* (Umbelliferae) from Nepal (I). A Histological Study of Leaves and the Botanical Origin of Tibetan Crude Drug TUNAK CHUNGA

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(Received on March 30, 1998)

To clarify histological diagnostics of the *Bupleurum* plants and the botanical origin of the crude drug TUNAK CHUNGA, leaves of eight Nepalese *Bupleurum* varieties were studied. The eight *Bupleurum* taxa were distinguished from each other based on the following anatomical features of leaves on the middle portion of stems: outlines of midrib and leaf margin in cross section, quantity of collenchyma tissue in leaf margin, number of oil canals in midrib and leaf blade, presence or absence of pappilae on epidermis, and number of stomata on the adaxial surface. Anatomical characteristics of each variety are described. The Tibetan crude drug TUNAK CHUNGA was identified as the whole plant of *B. falcatum* subsp. *falcatum* var. *gracillimum*, which is one of the most common *Bupleurum* plants in the alpine zone of the Nepal Himalayas, and is the first record as the botanical origin of Tibetan crude drug.

Bupleurum plants of the Family Umbelliferae are important as medicinal resources. Their roots are used as CHAI HU in the Chinese traditional medicine (Namba 1993), and the whole plant is also utilized in Chinese folk medicine and Tibetan medicine (Wu 1993, Xie 1990, Yang 1991). *Bupleurum* plants are distributed mainly in the northern hemisphere, and taxonomic treatment of Asian species has not been settled due to their morphological diversity (Hiroe 1958, Ohwi 1965, Shan and Sheh 1979, Mukherjee and Constance 1993). Especially, *B. falcatum* is quite variable, and Wolff (1910) reported four subspecies and 17 varieties under this species. In China the botanical origin of CHAI HU is complicated because of the great diversity in morphologically similar taxa. Though only two taxa, *B. chinense* DC. and *B.*

scorzonerifolium Willd., are cited in the Chinese pharmacopoeia (1995), actually roots of about 20 taxa are utilized as CHAI HU out of 36 taxa growing in China (Shan and Sheh 1979, Xie 1990). So far, it is not clear which taxon is the best material for the crude drug. Moreover no histological study on the genus has been done because of the plant's taxonomical uncertainty, and identification of the botanical origins of the crude drugs of *Bupleurum* derivatives in markets is not feasible.

Besides, in the alpine region of Western Nepal, the whole plants of *Bupleurum* species are used medicinally by Tibetans under the names of TAN GUN HA PU and TA QUIN (Namba et al. 1985). In 1995, the authors got a crude drug named TUNAK CHUNGA from a Tibetan hospital in Western Nepal, which

was assumed to be the whole plant of *Bupleurum* species.

Nepal has a various *Bupleurum* plants, and Cannon (1979) reported seven taxa from the Nepal Himalayas, five of which are common to those in China. Thus study of *Bupleurum* samples from Nepal should contribute to histological diagnostics and other pharmacognostical information to aid crude drug identification, especially of TUNAK CHUNGA. In this report, leaves of eight Nepalese *Bupleurum* taxa are histologically studied for these purposes.

Experimental

I. Materials

Experimental materials used in this study are kept at the Herbarium, the Herbal Garden of Faculty of Pharmaceutical Sciences, Kanazawa University (KANP), and some duplicates of the herbarium specimens are deposited at the Herbarium of the University of Tokyo (TI).

1. Plant specimens: About 120 herbarium samples were collected in 1991, 1993, 1994, 1995 and 1996 by M.Suzuki et al. (MS) and M.Mikage et al. (MM), in Middle, Western, and Far Western Nepal. First the materials were identified based on Wolff's (1910) and Mukherjee's (1993) studies into eight taxa: *B.candolii* Wall. ex DC., *B.lanceolatum* Wall. ex DC., *B.longicaule* Wall. ex DC. var. *himalayense* (Klotzsch) C.B.Clarke, *B.longicaule* Wall. ex DC. var. *strictum* C.B.Clarke, *B.hamiltonii* Balak, *B.falcatum* L. subsp. *marginatum* (Wall. ex DC.) H.Wolff, *B.falcatum* L. subsp. *falcatum* var. *gracillimum* (Klotzsch) H.Wolff, and *B.falcatum* L. subsp. *falcatum* var. *hoffmeisteri* (Klotzsch) H.Wolff. This covered all the principal taxa ever reported from Nepal (Cannon 1979).

2. The Tibetan crude drug TUNAK CHUNGA: Crude drug was obtained from a Tibetan doctor, Sonam Namgyal of the Muktinath Tradi-

tional Medical Centre, Jharkot, Mustang district, Dhawalagiri zone, Nepal, in September, 1995.

II. Histological analysis

1. Methods: Normal leaves of two to several specimens of each taxa are studied anatomically. Sample leaves are taken from the middle part of a stem, and transection and surface of the middle portion were observed with an optical microscope. A stomatal index, number of stomata in a square millimeter, and a palisade ratio, number of palisade parenchyma cells under one upper epidermal cell, were also measured from surface views.

2. General anatomical characters of leaves

Transection: Epidermis is one-cell layer and is covered with a thin cuticle. The outer cell wall is characteristically thickened in some taxa. Papillae develop in some species, and epidermal cells are triangular. Midrib projects remarkably to the abaxial side, and the adaxial surface is flat in some taxa and projected in others. Collenchyma tissue develops beneath the epidermis of the adaxial and abaxial sides of the midrib. Vascular bundle is collateral type. One to several schizogenous oil canals exist around the bundle. Taller specimen tends to have more oil canals. In leaf blades, one or two layers of palisade cells and spongy tissues are outstanding. Leaf blade swells near the leaf margin, and the margin is hooked or beaked in most taxa. The amount of collenchyma tissue in the leaf margin is characteristic to each taxa.

Surface view: Plentiful stomata scatter only on the abaxial surface in some taxa and on both surfaces in others. Papillae are observed on the epidermises of some taxa.

3. Characteristics of each taxon

i) *Bupleurum candolii* Wall. ex DC.

Materials: (Far West Nepal) Seti Zone, Bajura Distr., bet. Birseni and Porakya, MS9191291; Bajhang Distr., bet. Rasa and Roshadanda, MS9191427.

External morphology: Involucellate scales are rather large in size than those of other taxa

and exceed umbellets. Leaf blade is broadly linear to oblong, and lower cauline leaves cuneate at base and upper ones amplexicaul.

Leaf anatomy (Fig. 1-A): In the transection, midrib projects greatly to the abaxial side in U-

shape or semicircle, and adaxial surface is flat or slightly projected. Papillae develop especially on abaxial surface. Three to six oil canals of 15 to 20 μm in diameter surround the vascular bundle. Two to three oil canals are

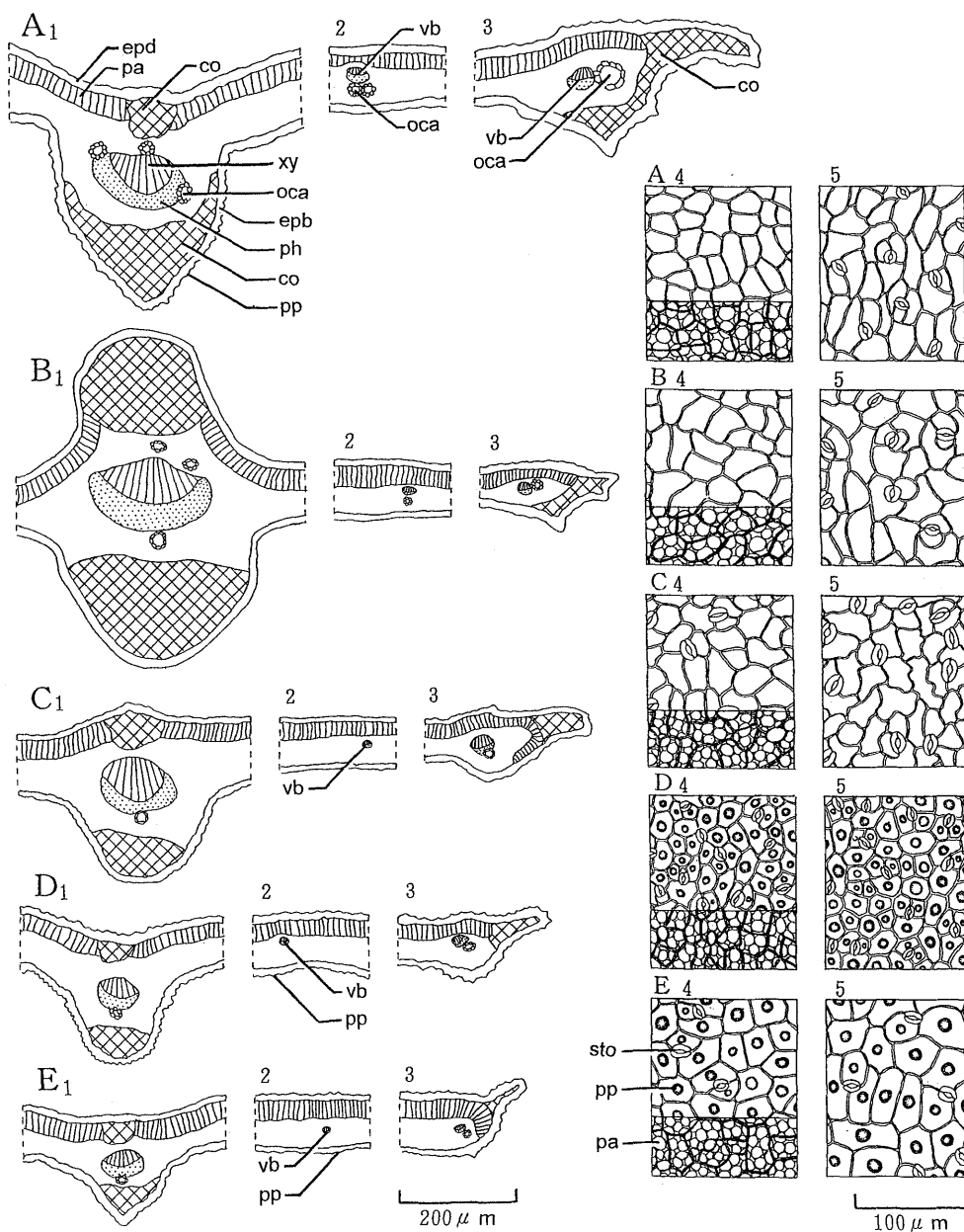


Fig. 1. Leaf anatomy of *Bupleurum* spp. from Nepal. A, *B. candollii*; B, *B. lanceolatum*; C, *B. longicaule* var. *himalayense*; D, *B. longicaule* var. *stricta*; E, *B. falcatum* subsp. *falcatum* var. *gracillimum*. (1, illustration of transection of midrib; 2, leaf blade; 3, leaf margin; 4, adaxial surface view; 5, abaxial surface view).

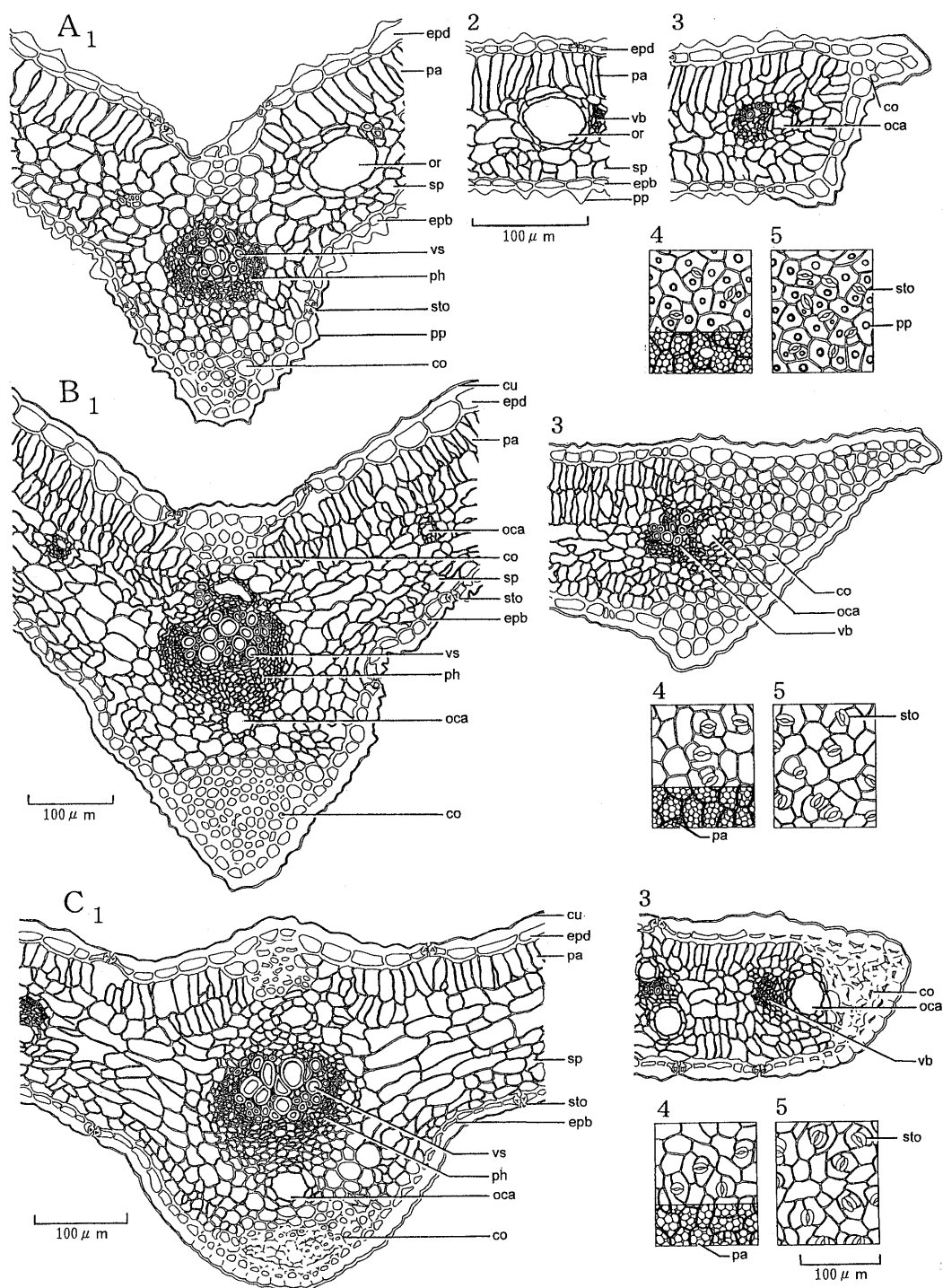


Fig. 2. Leaf anatomy of *Bupleurum* spp. from Nepal. A, *B. hamiltonii*; B, *B. falcatum* subsp. *marginatum*; C, *B. falcatum* subsp. *falcatum* var. *hoffmeisteri* (1, detailed drawing of the transection of midrib; 2, leaf blade; 3, leaf margin; 4, adaxial surface view; 5, abaxial surface view).

observed near the vascular bundle of lateral veins. Leaf blade swells characteristically near the margin. Considerable amount of stomata scatter on abaxial surface, while few appear along the veins on adaxial surface.

ii) *Bupleurum lanceolatum* Wall. ex DC.

Materials: (Far West Nepal) Seti Zone, Bajhang Distr., bet. Dhalaum and Rasa, MS9191411. (West Nepal) Dhawalagiri Zone, Myagdi Distr., bet. Lower Lete and Tatopani, MS9460464; Mustang Distr., bet. Ghasa and Tukche, MM9550236. Gandaki Zone, Gorkha Distr., bet. Ekle Ghar and Sardu Khola, MS9460085; bet. Sardu Khola and Lokpa, MS9460087; bet. Sardu Khola and Ripche, MS9460100; Manang Distr., bet. Bagarchhap and Chame, MS9460328.

External morphology: Involucellate scales are small and fall down before fruits mature. Leaf blade is lanceolate and apparently petiolate, and rhizomes elongate. The three attributes are characteristics of this species.

Leaf anatomy (Fig. 1-B): Midrib projects remarkably on the both sides. One to three oil canals measuring 20 to 25 μm in diameter surround the vascular bundle. Leaf blade swells slightly near the margin. Stomata scatter a lot on abaxial surface, while only few appear on adaxial surface along the veins. No papilla is recognized on the surfaces.

iii) *Bupleurum longicaule* Wall. ex DC. var. *himalayense* (Klotzsch) C.B. Clarke (= *B. rupestre* Edgew.)

Materials: (West Nepal) Gandaki Zone, Gorkha Distr., bet. Langdang Gompa and Torogompa Glacier, MS9460128; bet. Thangmanang Kharka and Sama Gompa, MS9460257; Manang Distr., bet. Yak Kharka and Thorung Phedi, MS9460404.

External morphology: Plant has radical leaves with a long petiole and a linear to lanceolate leaf blade. Leaves on the upper stem are narrow lanceolate with an amplexicaul leaf base. Leaves attached to the middle and lower stem are linear. Each involucre has 6 to 10 large scales exceeding umbellets. Florets are many, 15 to 50 in an involucre.

Leaf anatomy (Fig. 1-C): Midrib projects slightly to adaxial side and semicircularly to

abaxial side. One oil canal of 10 to 15 μm in diameter exists beneath the midrib vascular bundle. Papillae on surfaces are not prominent. Stomata scatter a lot on both adaxial and abaxial surfaces.

iv) *Bupleurum longicaule* Wall. ex DC. var. *strictum* C.B. Clarke

Materials: (Far West Nepal) Seti Zone, Bajura Distr., bet. Birseni and Porakya, MS9191292; bet. Pategaon and Badigaon, MS9191368; bet. Khaptad and Kandegaon, MS9191571.

External morphology: Though morphologically quite similar to var. *himalayense*, number of florets in an involucre is distinguishable smaller, 10 to 15, and each involucre has usually five small scales.

Leaf anatomy (Fig. 1-D): Though anatomically similar to var. *himalayense*, many conspicuous papillae exist on both surfaces.

v) *Bupleurum hamiltonii* Balak. (= *B. tenue* Buch.-Ham. ex D. Don)

Materials: (Far West Nepal) Seti Zone, Bajura Distr., bet. Tolebhir and Berma, MS9191247. (West Nepal) Dhawalagiri Zone, Myagdi Distr., bet. Ghorepani and Tatopani, MM9550140; bet. Dharapani and Muri, MM9681148; Mustang Distr., bet. Tatopani and Ghasa, MM9550185.

External morphology: Leaf is linear with many small orange dots of oil cavities. Each involucre has 4 to 6 florets.

Leaf anatomy (Fig. 2-A): Section is somewhat V-shaped. Midrib projects to the adaxial side in a V-shape. Many large oil cavities, about 50 μm in diameter, exist in the leaf blade. Stomata scatter on both surfaces. Papillae obviously develop on both surfaces.

vi) *Bupleurum falcatum* L. subsp. *marginatum* (Wall. ex DC.) H. Wolff (= *B. falcatum* L. var. *marginatum* (Wall. ex DC.) C.B. Clarke)

Materials: (Far West Nepal) Seti Zone, Bajhang Distr., bet. Rasa and Roshidanda, MS9191428. (West Nepal) Gandaki Zone, Gorkha Distr., bet. Ekle Ghar and Sardu Khola, MS9460063, 9470170; bet. Sardu Khola and Lokpa, MS9460094; bet. Landang Gompa and Dumje Thumje, MS9460170.

External morphology: Most involuclral and involucllate scales fall down before fruits mature. Leaf is linear with a clear white margin.

Leaf anatomy (Fig. 2-B): Midrib projects to the adaxial side in a V-shape. Two to three oil canals of 15 to 25 μm in diameter exist around the vascular bundle. Leaf margin is hooked or beaked and have remarkable collenchyma tissue consisting of 40 to 93 collenchyma cells. The outer cell wall of epidermal cells is obviously thickened. Stomata scatter on both surfaces. Pappilae on the surfaces are not prominent.

vii) *Bupleurum falcatum* L. subsp. *falcatum* var. *gracillimum* (Klotzsch) H. Wolff (= *B. gracillimum* Klotzsch)

Materials: (Far West Nepal) Seti Zone, Bajhang Distr., bet. Rasa and Roshidanda, MS9191426. (West Nepal) Dhawalagiri Zone, Mustang Distr., bet. Ghasa and Tukche, MM933101, 933105, 9550263; bet. Tatopani and Ghasa, MM9550231; vicinity of Jharkot, MM9550373, MS9460422; Gandaki Zone, Gorkha Distr., bet. Landang Gompa and Dumje Thumje, MS9460168; bet. Thorang Khora and Lhogaon, MS9460208; bet. Lhogaon and Thangmanang Kharka, MS9460210.

External morphology: Both involuclral and involucllate scales remain till fruits mature. Leaf shape is variable, somewhat short and narrow in comparison with the other varieties of *B. falcatum*.

Leaf anatomy (Fig. 1-E): The adaxial surface of the midrib is flat, slightly projected, or hollowed in some specimens. One oil canal of 15 to 25 μm in diameter exists near the vascular bundles of both midrib and lateral veins. Leaf margin is variable in shape, i.e., hooked, beaked, or somewhat acuminate. Collenchyma tissue develops a little at leaf margin. The outer cell wall of epidermal cells is somewhat thickened in some specimens and not in others. Stomata scatter on both surfaces. Pappilae on the surfaces are prominent in some specimens and not in others.

viii) *Bupleurum falcatum* L. subsp. *falcatum*

var. *hoffmeisteri* (Klotzsch) H. Wolff (= *B. falcatum* var. *hoffmeisteri* (Klotzsch) C.B. Clarke)

Materials: (West Nepal) Gandaki Zone, Gorkha Distr., bet. Deng and Thorang Khora, MS9460180b; bet. Thorang Khora and Lhogaon, MS9460209; Manang Distr., bet. Chame and Pisang, MS9460348, 9460358a; bet. Manang and Yak Kharka, MS9460369.

External morphology: Involuclral and involucllate scales are scarce. Leaf varies from linear to narrow lanceolate. Midrib and even the lateral veins project distinctively on both sides.

Leaf anatomy (Fig. 2-C): Midrib projects broad semicircularly on both sides. Three to five oil canals surround the vascular bundle of midrib. Lateral veins are also accompanied with two or three oil canals. Leaves scarcely swell near round or mucronate margin, characteristic of this variety. The outer cell wall of epidermal cells is obviously thickened. Stomata scatter on both surfaces. No papilla develops on the surfaces.

III. Tibetan crude drug TUNAK CHUNGA

External appearance: Crude drug obtained is dried whole plants with roots. Rhizomes has many branches. Stems are faciculate and about 30 cm in height. Leaves are sessile and leaf blade is linear, 2 to 4 cm long and 3 to 4 mm wide. Number of umbellula is 5 to 7 and florets are 7 to 12. Peduncles are 1 to 3 cm long and pedicels are about 1 mm long. Petals are yellow. Both involuclral and involucllate scales are elliptical, 2 to 5 mm and 2 to 3 mm long, respectively. Young fruits are about 3 mm long.

Leaf anatomy: Transverse section is somewhat V- or U-shaped. Midrib is deltoid, and the adaxial surface is almost flat or hollowed, 300 μm thick. One oil canal exists beneath the bundle. The adaxial surface of lateral vines is apparently hollowed. Leaf blade is 150–170 μm thick. Leaf margin is beaked and has scarce collenchyma cells. The outer cell wall of epidermal cells is somewhat thickened.

Table 1. Anatomical attributes of leaves of *Bupleurum* spp. from Nepal

| | <i>B. candolii</i> | <i>B. lanceolatum</i> | <i>B. longicaule</i> var. <i>himalayense</i> | var. <i>strictum</i> | <i>B. hamiltonii</i> | <i>B. falcatum</i> subsp. <i>marginatum</i> | subsp. <i>falcatum</i> var. <i>gracillimum</i> | subsp. <i>falcatum</i> var. <i>hoffmeisteri</i> |
|--|-----------------------------|-------------------------------|---|----------------------|----------------------|--|---|--|
| (Cross Section) | | | | | | | | |
| Midrib | | | | | | | | |
| Projection upper side* | —~+ | ++ | + | —~+ | — | —~+ | —~+ | + |
| lower side | semicircular to U-shaped | semicircular to pot-shaped | semicircular to broad V-shaped | semicircular | V-shaped | V-shaped | V-shaped | semicircular |
| Thickness (μm) | 300~410 | 300~730 | 250~300 | 250~290 | 240~330 | 400~570 | 220~390 | 280~500 |
| Diameter of vessel (μm) | 10~20 | 10~25 | 10~20 | 10~20 | 10~22 | 10~20 | 15~25 | 15~30 |
| Oil canal | | | | | | | | |
| Number | 3~6 | 1~3 | 1~2 | 1~2 | 1 | 2~3 | 1 | 3~5 |
| Diameter (μm) | 15~20 | 20~25 | 10~15 | 8~10 | 15~20 | 15~25 | 15~25 | 25~55 |
| Lateral vein | | | | | | | | |
| Number of oil canal | 2~3 | 1~2 | 1 | 1 | 1 | 2 | 1 | 2~3 |
| Leaf blade | | | | | | | | |
| Thickness (μm) | 90~110 | 100~150 | 90~110 | 100~110 | 110~150 | 160~200 | 90~180 | 170~220 |
| Oil cavity | — | — | — | — | + | — | — | — |
| Leaf margin | | | | | | | | |
| Swelling near margin | ++ | + | + | ±~+ | ±~+ | +++ | ±~+ | —~+ |
| Shape | hooked or beaked | hooked or beaked | hooked | hooked | hooked or beaked | hooked or beaked | hooked, beaked or acuminate | round or mucronate |
| Collenchyma cells** | ++++~++++ | +~++++ | +~+++ | +~+++ | + | ++++~+++++ | +~+++ | +~+++ |
| Epidermis | | | | | | | | |
| Thickening of outer wall | — | — | — | — | —~+ | +++ | —~+++ | +++ |
| Pappila | + | — | —~+ | +++~++++ | +++ | + | + | — |
| (Surface View) | | | | | | | | |
| Stomatal index (number/mm ²) | | | | | | | | |
| Upper surface | few along vine | few along vine | 300~500 | 250~390 | 160~430 | 190~330 | 30~350 | 230~380 |
| Lower surface | 300~400 | 350~380 | 350~380 | 200~280 | 150~470 | 360~410 | 160~500 | 200~380 |
| Palisade ratio | 2~4 | 4~5 | 3~5 | 4~6 | 5~12 | 8~12 | 3~7 | 5~8 |

*—: flat or V-shaped (hollowed). +: project slightly. ++: project semicircularly.

**+: less than 10 cells. ++: 11 to 20 cells. +++: 21 to 30 cells. ++++: 31 to 40 cells. +++++: more than 41 cells.

Pappilae develop on both surfaces.

The botanical origin: The crude drug was identified as a whole plant of *B. falcatum* subsp. *falcatum* var. *gracillimum* both from external morphology and leaf anatomy.

Results and Discussions

1. The eight *Bupleurum* taxa from Nepal Himalaya could be anatomically distinguished from each other. Characteristic features were recognized in cross sections and surface views of leaves on the middle portion of stems; they were shape of midrib and leaf margin in cross section, quantity of collenchyma tissue in leaf margin, numbers of oil canals in the midrib and leaf blade, presence or absence of pappila on epidermises, and numbers of stomata on adaxial surface (Table 1).

2. The Tibetan crude drug TUNAK CHUNGA, obtained from a hospital in Jarkot, Mustang District of Nepal, was identified as the whole plant of *B. falcatum* subsp. *falcatum* var. *gracillimum*. The name TUNAK CHUNGA has not appeared in any literature of Tibetan medicine up to now. Also this taxon has not recorded in any Tibetan drug's literature so far as a botanical origin. This is one of the most common *Bupleurum* plants in the alpine zone of the Nepal Himalayas.

3. Plant taxonomically, Mukherjee and Constance (1993) recognized two species *B. rupestre* Edgew. and *B. longicaule* DC., instead of *B. longicaule* var. *himalayense* and *B. longicaule* var. *strictum* used in this study, based on the number of flowers and bracteoles in an umbel and the fruit shape. In addition to this, we noticed difference in the fruiting seasons of these two taxa, var. *himalayense* fruiting 2 to 4 weeks later than var. *strictum* within the same habitat. We agreed with Mukherjee and Constance (1993) in recognizing two distinct taxa, and regarded them as the varieties of *B. longicaule* in this report, based on the leaf anatomical similarity of the two except for the

papillae on leaf surfaces of var. *strictum*.

4. In Yunnan Province, China, the whole plants of *B. hamiltonii* and *B. falcatum* subsp. *marginatum* are used as a kind of CHAI FU (Wu 1993, Xie 1990). Both plants are rich in oily substances in their leaves. From ancient times, oily CHAI FU has been considered to be of good quality. Though CHAI FU is essentially roots of *Bupleurum* plants, utilization of whole plants of these two varieties may be due to an abundance of oily substances contained in the aerial part.

Experimental materials in this study were mainly collected through the expeditions supported by Monbusho Grants-in-Aid for International Scientific Research: Field Research (MM, No. 07041132; M. Suzuki, Nos. 06041043, 03041035) from the Ministry of Education, Science and Culture, Japan. We are also grateful to Dr. H. Ohba for the loan of specimens from the Herbarium, the University of Tokyo (TI).

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List of abbreviations: **co**, collenchyma cell or collenchyma tissue; **cu**, cuticle; **epb**, epidermis of abaxial side; **epd**, epidermis of adaxial side; **oca**, oil canal; **pa**, palisade parenchyma cell or palisade tissue; **ph**, phloem; **pp**, papilla; **sp**, spongy tissue; **sto**, stoma; **vb**, vascular bundle; **vs**, spiral vessel; **xy**, xylem.

御影雅幸, 毛塚重行: ネパール産ミシマサイコ属植物の研究(1). 葉の組織学的研究ならびにチベット薬物 TUNAK CHUNGA の原植物

ネパール産のセリ科ミシマサイコ属 (*Bupleurum*) 8 分類群の葉を比較組織学的に検討し, 本属の組織分類学的な要素を明らかにするとともに, ネパールの高山帯で薬用として利用されているチベット薬物 TUNAK CHUNGA の基源解明を試みた. その結果, 組織学的には茎の中央部付近の葉において, 横切面における主脈部や葉縁部の形, 厚角組織の発達状態, 油道の存在数, 乳状突

起の有無や上面の気孔の分布数などの形質で全種を分類することが可能であった (Table 1). また TUNAK CHUNGA の基源は, ネパール高山帯の本属植物では資源的にもっとも豊富な *B. falcatum* subsp. *falcatum* var. *gracillimum* の全草であることが明らかになった. 本種はチベット薬物の原植物としての初めての記録である. (金沢大学薬学部)